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Original Contribution

Cytomegalovirus Infection and the Risk of Mortality and Frailty in Older Women: A Prospective Observational Cohort Study

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Cytomegalovirus (CMV), a prevalent pathogen, causes severe disease in immunocompromised humans. However, present understanding is limited regarding the long-term clinical effect of persistent CMV infection in immunocompetent adults. The authors conducted a prospective observational cohort study (1992–2002) of 635 community-dwelling women in Baltimore, Maryland, aged 70–79 years in the Women's Health and Aging Studies to examine the effect of CMV infection on the risk of frailty, a common geriatric syndrome, and mortality in older women. The effect of baseline serum CMV antibody (immunoglobulin G) concentration on the risk of 3-year incident frailty, defined by using a 5-component measure, and 5-year mortality was examined with Cox proportional hazards models. Compared with those who were CMV seronegative, women in the highest quartile of CMV antibody concentration had a greater incidence of frailty (hazard ratio = 3.46, 95% confidence interval: 1.45, 8.27) and mortality (hazard ratio = 3.81, 95% confidence interval: 1.64, 8.83). After adjustment for potential confounders, CMV antibody concentration in the highest quartile independently increased the risk of 5-year mortality (hazard ratio = 2.79, 95% confidence interval: 1.22, 6.40). Better understanding of the long-term clinical consequences of CMV infection in immunocompetent humans is needed to guide public health efforts for this widely prevalent infection.

antibodies, viral; cytomegalovirus; frail elderly; immunoglobulin G; inflammation; interleukin-6; mortality; virus latency

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IL-6, interleukin-6.

Human cytomegalovirus (CMV) is a prevalent herpesvirus best known for causing congenital infection and severe opportunistic disease in immunocompromised persons (1). Primary CMV infection in most immunocompetent adults is asymptomatic or nonspecifically symptomatic, leaving no clinically observable trace of the infection except seroconversion (1). Acquisition of specific humoral and cellular immunity does not eradicate the virus but is followed by persistent infection in undifferentiated monocytes (2) and endothelial cells (3). Worldwide CMV seroprevalence can range from 52% to 99% among women of reproductive age (4). In the United States, 54% of adults 30–39 years of age and 91% of adults 80 years of age or older are CMV seropositive and thus harbor the virus (5).

Epidemiologic associations have been reported between CMV and cardiovascular disease (6–8), functional impairment (9), cognitive decline (10), prolonged hospitalization (11), and frailty (12), a common geriatric syndrome (13) that independently predicts adverse outcomes (14). However, the long-term effect of CMV infection in immunocompetent adults remains unclear, in part because of the cross-sectional nature of these studies (7, 9, 12) and conflicting results (6, 15). Despite the previous report of an association between CMV infection and mortality in immunocompetent older adults, generalizability was limited by the nature of

the study population, which included only those individuals with cardiovascular disease (16). Given the high prevalence of CMV infection worldwide and its potential implications on long-term clinical outcomes, we conducted a prospective cohort study in the Women's Health and Aging Studies I and II to determine the effect of persistent CMV infection on incident frailty and mortality in immunocompetent older women. Since CMV antibody, rather than viral DNA, is detectable in the serum of immunocompetent persons and may potentially correlate with cumulative viral burden (17, 18), we hypothesized that higher CMV antibody concentration would be associated with a higher risk of frailty and mortality.

MATERIALS AND METHODS

Study design

This study involved 635 older women who participated in the Women's Health and Aging Studies I and II, 2 complementary prospective observational studies begun in 1992 and 1994, respectively, of community-dwelling women in Baltimore, Maryland (19, 20). Both cohorts were obtained from the same sampling frame, the Health Care Financing Administration's Medicare eligibility lists for 12 contiguous zip code areas in Baltimore. Women's Health and Aging Study I enrolled 1,002 women aged 65 years or older who had self-reported difficulty in 2 or more of 4 domains of physical function. Women's Health and Aging Study II enrolled 436 women aged 70–79 years who had difficulty in no more than 1 domain. Details on the study methods and sampling design of these studies have been published elsewhere (19, 20) and are summarized in Web Appendix 1, the first of 8 Web Appendixes posted on the *Journal*'s website (http://aje.oxfordjournals.org/).

The research protocols were approved by the Johns Hopkins University's institutional review board. Informed consent was obtained from all participants.

This study consisted of a cross-sectional component, in which we examined the dose-response relation between baseline serum CMV immunoglobulin G (IgG) antibody concentration and the risk of prevalent frailty, and a longitudinal component, in which we examined the relation between baseline CMV IgG concentration and the risk of 3-year incident frailty and 5-year all-cause mortality. The population for the present study was derived by combining individuals from the Women's Health and Aging Studies I and II cohorts aged 70–79 years at the baseline visit. Of the 829 women in this age range, 172 were excluded because of lack of baseline serum specimens, 2 for not being black or white, and 20 because baseline covariates were missing. For the cross-sectional analysis, after 3 participants with a missing baseline frailty measure were excluded, the final sample size was 632. For the longitudinal incident frailty analysis, we excluded an additional 78 participants because they were frail at baseline, 37 because a blood sample was not obtained within 12 months of the baseline frailty assessment, and 35 because no frailty data were available after baseline blood draw, resulting in a final sample size of 482. For the mortality analysis, a sample size of 635 was derived in the same manner as for the cross-sectional sample, except that participants missing a baseline frailty measure were not excluded.

CMV antibody and interleukin-6 measurement

CMV IgG concentration was measured in stored (−70°C) baseline serum, originally obtained from 1992 to 1995 for the Women's Health and Aging Study I and from 1994 to 1996 for the Women's Health and Aging Study II, using a commercial enzyme-linked immunosorbent assay kit (GenWay Biotech, San Diego, California). Seropositivity for CMV was defined, according to manufacturer's instructions, as a serum IgG concentration of >1.2 IU/mL. Additional details are provided in Web Appendix 2. Plasma interleukin-6 (IL-6) concentrations were measured by using a commercial enzyme-linked immunosorbent assay kit (Quantikine Human IL-6; R&D Systems, Minneapolis, Minnesota). All assays were performed in a masked fashion.

Outcome measurement

Frailty was defined by using a 5-component measure originally proposed in the Cardiovascular Health Study (14) and subsequently validated in the Women's Health and Aging Study (21): 1) weight loss, 2) exhaustion, 3) low energy expenditure, 4) slowness, and 5) weakness. Detailed methods of defining each component are described in Web Appendix 3. Participants meeting 3 or more of these criteria were classified as frail, those meeting 1 or 2 as prefrail, and those meeting none as nonfrail. This definition has been shown to be consistent with the conceptualization of frailty as a medical syndrome distinct from comorbidity and disability (14, 21). Incident frailty was defined as the onset of frailty in a participant who was nonfrail or prefrail at baseline.

Vital status was obtained through follow-up interviews with proxies, obituaries, and matching with the National Death Index during a median follow-up of 60 months. Death certificates were obtained for all but 6 of the women who died.

Statistical analysis

Study participants' baseline characteristics were compared across categories of CMV antibody concentration by using analysis of variance or the nonparametric equality-of-medians test for continuous variables with a normal or a skewed distribution, respectively, and the chi-square test for binary variables. For the cross-sectional analysis, a multinomial logistic regression model was constructed to control for potential confounders: age, race, completion of a high school education, coverage by private medical insurance, history of smoking, cardiovascular disease, diabetes mellitus, and plasma IL-6 concentration. Because IL-6, an inflammatory cytokine, has been shown to significantly modify the relation between CMV and prevalent frailty (12), an interaction term between IL-6 and CMV antibody concentration was included in the final model. The definition of and rationale for choosing the covariates are provided in Web Appendix 4.

For the longitudinal analysis, Cox proportional hazards models were fit to investigate the association between baseline CMV antibody concentration and time until frailty or death, controlling for baseline covariates as in the cross-sectional analysis. Average follow-up duration was 3 years for frailty status and 5 years for mortality. Tests of the proportional hazards assumption based on Schoenfeld residuals indicated no violation of the assumption for all covariates (22, 23). Plotting martingale residuals from the Cox model without covariates against each covariate produced approximately linear smooth curves and validated the functional form for the covariates entered into the models. Since the interaction between CMV antibody and IL-6 concentration was not significant in both the mortality and incident frailty models, we constructed final longitudinal models without the interaction but additionally constructed models with the interaction term for consistency with the cross-sectional analysis. In sensitivity analyses, we examined the robustness of the longitudinal incident frailty and mortality results by excluding participants with baseline cardiovascular disease and categorizing CMV antibody concentration using 3 alternative schemes.

All *P* values were derived from 2-tailed tests. Analyses were conducted by using Stata version 10.0 software (StataCorp, College Station, Texas). To appropriately reference inferences derived from the study participants back to the sampling population of community-dwelling women aged 70–79 years, we used study-specific probability weights to weight each woman's data to reflect the number of women she represented in the sampling frame. Construction of the weights has been described previously (24). To account for missingness in CMV antibody data, weights were further multiplied by the inverse proportion of CMV antibody data missingness, separately for each Women's Health and Aging Study cohort. Study-specific probability weights were incorporated in all descriptive and regression analyses by using the survey weighting capability of Stata software. All population percentages reported herein were thus derived.

RESULTS

Of the 635 participants, 92 had a baseline serum CMV IgG concentration of $\leq$1.2 IU/mL, reflecting no prior CMV infection, and 543 had a concentration of $>$1.2 IU/mL, reflecting persistent infection. Participants with evidence of persistent CMV infection were further categorized into 4 groups according to quartiles of CMV antibody concentration (IU/mL): 1.21–10.16, 10.17–14.55, 14.56–18.16, and 18.17–150. As expected from previously reported CMV seroepidemiologic data from the US population (5), participants with different serum CMV antibody concentrations differed in age, race, completion of a high school education, and coverage by private medical insurance (Table 1). Women with higher CMV antibody concentrations were more likely to be older, be black, have not completed a high school education, have no private medical insurance, have diabetes, have osteoarthritis, and have lower Mini-Mental State Examination scores.

At baseline, 263 (44.1%) participants were nonfrail, 296 (45.2%) were prefrail, and 73 (10.7%) were frail. The prevalence of frailty increased with higher CMV IgG concentrations, being 5.6% among those who were seronegative and 18.4% among those who were in the highest quartile of CMV antibody concentration (chi-square test: $P = 0.003$; Table 2).

The independent association between CMV antibody concentration and prevalent frailty in community-dwelling older women was assessed by controlling for potential confounders and modeling for effect modification by IL-6. At the lower 2 tertiles of plasma IL-6 concentration, there was a suggestion of an association between the highest CMV antibody concentration and frailty, the magnitude of association being greater at the second than the first IL-6 tertile, but the associations were not significant (Table 3). At the highest tertile of IL-6 concentration, we found an association between the highest quartile of CMV antibody concentration and frailty (adjusted odds ratio = 5.18, 95% confidence interval: 0.99, 27.05), the magnitude of association being greater than for the lower IL-6 tertiles. Similar trends were noted in the association between the highest CMV antibody concentration and prefrailty, except that the magnitudes of association were lower than those for frailty. Additional analysis of the dose-response relation between CMV infection and prevalent frailty is provided in Web Appendix 5.

Among participants at risk, 80 (16.6%) developed incident frailty during 3 years of follow-up and 91 (13.2%) died during 5 years of follow-up. Compared with those who were CMV seronegative at baseline, women in the highest quartile of CMV antibody concentration had a greater incidence of frailty (91.8 vs. 29.6 events per 1,000 person-years; hazard ratio = 3.46, 95% confidence interval: 1.45, 8.27; Table 4). Compared with those who were CMV seronegative at baseline, older women in the highest quartile of CMV antibody concentration had a greater mortality rate (52.8 vs. 14.0 events per 1,000 person-years; hazard ratio = 3.81, 95% confidence interval: 1.64, 8.83). Although the hazard ratios for women in the lower 3 quartiles of CMV antibody concentration suggested higher risks for incident frailty and mortality compared with CMV-seronegative women, the 95% confidence intervals all contained the null value. The causes of death are summarized in Web Appendix 6.

To assess the independent effect of CMV infection on incident frailty and mortality, we adjusted for potential confounders in Cox proportional hazards models. Although the adjusted hazard ratios for women in the lower 3 quartiles of CMV antibody concentration suggested higher risks for incident frailty and mortality compared with CMV seronegative women, the 95% confidence interval contained the null value, as in the unadjusted models (Table 4). In contrast, the highest quartile of CMV antibody concentration independently predicted a greater 5-year mortality rate (adjusted hazard ratio = 2.79, 95% confidence interval: 1.22, 6.40). Although there was a suggestion of an independent association between the highest quartile of CMV antibody concentration and 3-year incident frailty, the 95% confidence interval of the hazard ratio contained the null value (hazard ratio = 2.26, 95% confidence interval: 0.89, 5.75). Results

Table 1. Baseline Characteristics of Study Participants in the Analytic Sample Selected From the Women's Health and Aging Studies I and II, Baltimore, Maryland, 1992–2002

Characteristic[a]	All Participants (n = 635)	CMV IgG Antibody Concentration, IU/mL[b]				
		≤1.20 (n = 92)	1.21–10.16 (n = 141)	10.17–14.55 (n = 128)	14.56–18.16 (n = 130)	18.17–150 (n = 144)
Mean age, years*	73.9	73.3	73.7	74.1	74.2	74.3
White race, %*	76.8	90.2	76.0	77.3	76.4	68.7
Completion of a high school education, %**	58.2	82.9	53.7	60.0	54.1	48.3
Coverage by private medical insurance, %*	81.1	91.3	84.4	78.4	81.5	73.5
Current or former smoking, %	49.8	62.5	42.7	45.2	48.4	54.2
Diabetes mellitus, %*	14.3	9.9	10.7	12.8	14.5	22.0
Cardiovascular disease, %	46.0	40.6	41.8	45.0	46.0	54.7
Osteoarthritis, %*	72.5	70.1	70.4	71.6	83.2	66.3
Osteoporosis, %	42.9	40.1	38.4	46.2	42.0	46.8
Pulmonary disease, %	38.3	42.6	37.9	39.4	36.0	37.3
Median Mini-Mental State Examination score	29	30	28	29	28	28
Median plasma IL-6 concentration, pg/mL	2.7	2.6	2.8	2.7	2.8	2.8

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G; IL-6, interleukin-6.

* $P < 0.05$; ** $P < 0.001$ (P for difference across categories of CMV IgG antibody concentration).

[a] Percentages were those among all participants or within each category of CMV antibody concentration and were calculated with the use of study-specific probability weights.

[b] Baseline CMV IgG antibody concentration of >1.20 IU/mL was considered seropositive, reflecting prior CMV infection. The categorization of antibody concentration grouped subjects into those who were CMV seronegative and those who were in the first, second, third, and fourth quartiles, respectively, of the seropositive range of CMV antibody concentration.

from additional Cox models that included an interaction term between CMV antibody and IL-6 concentration are provided in Web Appendix 7.

We performed sensitivity analyses to assess the robustness of these results, by categorizing CMV antibody concentration in 3 alternative schemes (Web Appendix 8). The association between the highest CMV antibody concentration and greater mortality risk continued to be observed when antibody concentration was categorized differently. However, dichotomizing antibody concentration (seronegative vs. seropositive) resulted in loss of information regarding the independent association between the highest CMV antibody concentrations and mortality (Web Appendix 8, Web Table 6).

Given the known association between CMV infection and cardiovascular disease (8), we performed additional

Table 2. Prevalence of Frailty States by CMV IgG Antibody Concentration, Women's Health and Aging Studies I and II, Baltimore, Maryland, 1992–2002

Frailty Status, %[a]	All Participants (n = 632)	CMV IgG Antibody Concentration, IU/mL[b]				
		≤1.20 (n = 92)	1.21–10.16 (n = 140)	10.17–14.55 (n = 128)	14.56–18.16 (n = 129)	18.17–150 (n = 143)
Nonfrail	44.1	58.3	47.8	43.4	45.2	30.6
Prefrail	45.2	36.1	46.1	44.5	45.3	51.0
Frail	10.7	5.6	6.1	12.1	9.5	18.4

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G.

[a] Prevalent frailty states were measured at baseline. Percentages were those among all participants or within each category of CMV antibody concentration and were calculated with the use of study-specific probability weights.

[b] Baseline CMV IgG antibody concentration of >1.20 IU/mL was considered seropositive, reflecting prior CMV infection.

Table 3. Adjusted Odds Ratios[a] for Prevalent Frailty States by CMV IgG Antibody Concentration, Effect-Modified by IL-6 Concentration, Women's Health and Aging Studies I and II, Baltimore, Maryland, 1992–2002

Tertile of Plasma IL-6 Concentration[b]	Frailty Status	CMV IgG Antibody Concentration, IU/mL[c]								
		≤1.20 (*n* = 92)	1.21–10.16 (*n* = 140)		10.17–14.55 (*n* = 128)		14.56–18.16 (*n* = 129)		18.17–150 (*n* = 143)	
		OR	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
First	Prefrail	1.00	1.20	0.50, 2.86	1.47	0.61, 3.51	0.79	0.31, 2.01	1.10	0.47, 2.55
	Frail	1.00	1.44	0.29, 7.11	2.24	0.46, 10.86	0.62	0.12, 3.16	1.77	0.38, 8.32
Second	Prefrail	1.00	1.30	0.66, 2.54	1.39	0.72, 2.67	1.21	0.62, 2.38	1.84	0.94, 3.57
	Frail	1.00	0.59	0.15, 2.34	1.72	0.52, 5.69	1.09	0.33, 3.56	2.92	0.95, 9.04
Third	Prefrail	1.00	1.42	0.59, 3.41	1.31	0.57, 2.97	1.96	0.86, 4.44	3.30	1.41, 7.70
	Frail	1.00	0.21	0.02, 1.87	1.28	0.22, 7.46	2.06	0.38, 11.17	5.18	0.99, 27.05

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IgG, immunoglobulin G; IL-6, interleukin-6; OR, odds ratio.

[a] Odds ratios were derived from a multinomial logistic regression model, adjusting for age, race, high school education, coverage by private medical insurance, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), and diabetes mellitus, and modeling for effect modification by plasma IL-6 concentration. Comparisons were made with participants whose CMV IgG antibody concentration was ≤1.20 IU/mL. The reference category was "nonfrail."

[b] Plasma IL-6 concentration was modeled as a continuous variable. Odds ratios were derived by setting IL-6 to the midpoints (medians) of each indicated tertile, the respective values of which were 1.62, 2.69, and 4.81 pg/mL.

[c] A baseline CMV IgG antibody concentration of >1.20 IU/mL was considered seropositive, reflecting prior CMV infection.

sensitivity analyses to assess the contribution of cardiovascular disease to the higher incidence of frailty and mortality in participants with a high serum CMV antibody concentration. After excluding participants with baseline cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), we repeated the incident frailty and mortality analyses, in which the sample sizes were consequently reduced by 38% and 46%, respectively (Table 5). Cardiovascular-disease-free older women in the highest quartile of CMV antibody concentration, compared with CMV-seronegative women, still had higher incident frailty and mortality rates. After adjusting for potential confounders, there remained a suggestion of higher risks of incident frailty and mortality for women in the highest quartile of CMV antibody concentration, although the 95% confidence intervals for the adjusted hazard ratios were now broader (Table 5; compare with Table 4), as would be expected from a 38%–46% decrease in sample size.

DISCUSSION

In this prospective study of community-dwelling older women aged 70–79 years, those in the highest quartile of CMV IgG antibody concentration had higher risks of 3-year incident frailty and 5-year mortality than those who were CMV seronegative. After adjustment for potential confounders, the highest quartile of CMV antibody concentration independently predicted a higher risk of 5-year mortality.

Given the high prevalence of CMV infection worldwide, a better understanding of its long-term clinical effect in immunocompetent adults has an important public health impact. A previous study, also nested in the Women's Health and Aging Study, showed an association between CMV IgG seropositivity and prevalent frailty (12), the risk factors for which include older age, lower educational level, lower income, cigarette smoking, arthritis, chronic obstructive pulmonary disease, hypertension, diabetes, and cardiovascular disease (14, 25–27). However, the dichotomous antibody measurement precluded further examination of any dose-response effect of CMV infection. Our study clarifies that the previously reported association between CMV and frailty (12) is strongest at the highest CMV antibody and highest IL-6 concentrations.

In immunocompetent humans, CMV maintains a lifelong infection through immunomodulatory mechanisms that evade viral clearance by the hosts (28). It is unclear whether any potential effect of CMV infection on frailty or mortality risk for older women is the culmination of pathologic cellular and end-organ changes accumulated over life. To our knowledge, no definitive evidence exists to support the hypothesis that more frequent CMV reactivations, and thus more frequent antigenic stimuli, result in higher antibody titers. The observations that antibody titers increase over time in CMV shedders (17) and are generally higher in older persons (18, 29) suggest that serum CMV IgG concentration could be a function of the cumulative frequency of viral reactivation. Therefore, it is conceivable that individuals who have higher CMV antibody concentrations have experienced either more frequent viral reactivations or a longer infection. A higher cumulative frequency of viral reactivation could in turn represent a proportionately higher cumulative inflammatory burden and pathologic insult, through such mechanisms as atherogenesis (30) and local host-cell damage by a potent viral proinflammatory chemokine (31).

Table 4. Incident Frailty and Mortality Rates by CMV IgG Antibody Concentration, Women's Health and Aging Studies I and II, Baltimore, Maryland, 1992–2002

	All Participants	CMV IgG Antibody Concentration, IU/mL[a]				
		≤1.20	1.21–10.16	10.17–14.55	14.56–18.16	18.17–150
Incident frailty (3 years)						
No. at risk	482	73	117	101	93	98
No. of events	80	7	23	14	13	23
Incidence per 1,000 person-years	60.1	29.6	69.1	52.0	51.9	91.8
95% CI	48.3, 75.6	14.3, 71.9	46.4, 107.1	30.3, 97.3	30.8, 94.6	62.0, 141.5
Unadjusted hazard ratio		1.0	2.33	1.82	1.72	3.46
95% CI			0.98, 5.56	0.70, 4.70	0.67, 4.43	1.45, 8.27
Adjusted hazard ratio*,[b]		1.0	2.00	1.44	1.27	2.26
95% CI			0.79, 5.07	0.53, 3.95	0.46, 3.51	0.89, 5.75
All-cause mortality (5 years)						
No. at risk	635	92	141	128	130	144
No. of events	91	7	18	17	14	35
Incidence per 1,000 person-years	28.0	14.0	23.7	27.5	19.1	52.8
95% CI	22.7, 35.0	6.7, 34.2	14.9, 39.5	16.8, 47.9	11.3, 34.7	37.9, 75.4
Unadjusted hazard ratio		1.0	1.68	1.97	1.36	3.81
95% CI			0.68, 4.16	0.78, 4.94	0.53, 3.50	1.64, 8.83
Adjusted hazard ratio**,[b]		1.0	1.95	1.87	1.29	2.79
95% CI			0.81, 4.68	0.75, 4.65	0.52, 3.24	1.22, 6.40

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IgG, immunoglobulin G.

* $P = 0.34$; ** $P < 0.05$ (P by likelihood ratio test).

[a] A baseline CMV IgG antibody concentration of >1.20 IU/mL was considered seropositive, reflecting prior CMV infection.

[b] Hazard ratios were derived from Cox proportional hazards models, adjusting for age, race, high school education, coverage by private medical insurance, pack-years of smoking, cardiovascular disease (angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke), diabetes mellitus, and plasma interleukin-6 concentration. Comparisons were made with participants whose CMV IgG antibody concentration was ≤1.20 IU/mL.

The effect of these insults integrated over time could then culminate in accelerated host death or the manifestation of frailty, which has been hypothesized to be the aggregate expression of subthreshold damages to multiple physiologic systems (32).

The modifying effect of plasma IL-6 concentration on the relation between CMV infection and frailty suggests that any potential contribution of CMV to the pathogenesis of frailty is likely grounded in synergistic interaction with IL-6 production, consistent with the modifying effect of IL-6 on the association between CMV and cardiac mortality (33). CMV infection increases IL-6 gene expression and production in peripheral blood mononuclear cells (34). Our results suggest that the magnitudes of association between the highest CMV antibody concentration and prevalent frailty are higher at higher IL-6 concentrations. It can be envisioned, therefore, that any potential pathologic effect that CMV might exert on the development of host frailty requires the synergy of IL-6 production, induced by either inflammatory activation of non-CMV origin (32) or CMV itself (31). This requirement for high IL-6 concentration, and thus for the host genetic, physiologic, and environmental factors that might underlie elevation of this proinflammatory cytokine (35), could explain why there is not a one-to-one correspondence between the highly prevalent CMV infection and frailty in older women. The lack of a statistically significant interaction between IL-6 and CMV in incident frailty and mortality analyses might result from inadequate power for our longitudinal samples, since there was a suggestion of effect modification by IL-6 on the associations of CMV with incident frailty and mortality.

It is possible that the higher mortality rate among women with the highest CMV antibody concentration could be mediated partially or completely by cardiovascular disease,

Table 5. Incident Frailty and Mortality Rates by CMV IgG Antibody Concentration, Excluding Participants With Baseline Cardiovascular Disease,[a] Women's Health and Aging Studies I and II, Baltimore, Maryland, 1992–2002

	All Participants	CMV IgG Antibody Concentration, IU/mL[b]				
		≤1.20	1.21–10.16	10.17–14.55	14.56–18.16	18.17–150
Incident frailty (3 years)						
No. at risk	299	49	77	64	56	53
No. of events	39	3	13	8	7	8
Incidence per 1,000 person-years	47.1	16.4	58.9	47.6	43.8	63.4
95% CI	34.4, 66.2	5.1, 79.8	34.8, 107.5	22.5, 118.9	21.4, 104.5	32.3, 142.0
Hazard ratio		1.0	3.55	2.94	2.64	4.47
95% CI			1.01, 12.46	0.76, 11.41	0.69, 10.09	1.19, 16.74
Adjusted hazard ratio[c]		1.0	2.52	1.72	1.43	2.37
95% CI			0.68, 9.37	0.40, 7.41	0.33, 6.30	0.58, 9.58
All-cause mortality (5 years)						
No. at risk	344	56	85	70	71	62
No. of events	28	3	5	3	8	9
Incidence per 1,000 person-years	17.5	8.8	11.9	13.4	21.0	33.2
95% CI	11.9, 26.7	2.6, 45.7	5.0, 35.5	3.8, 80.0	10.4, 48.5	16.9, 74.5
Hazard ratio		1.0	1.36	1.53	2.4	3.83
95% CI			0.31, 5.95	0.29, 8.09	0.60, 9.64	0.97, 15.08
Adjusted hazard ratio[c]		1.0	1.29	1.05	1.60	2.46
95% CI			0.28, 5.96	0.18, 6.26	0.39, 6.61	0.65, 9.37

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; IgG, immunoglobulin G.

[a] Cardiovascular disease was defined as the presence of any of the following: angina, myocardial infarction, congestive heart failure, peripheral artery disease, or stroke.

[b] A baseline CMV IgG antibody concentration of >1.20 IU/mL was considered seropositive, reflecting prior CMV infection.

[c] Hazard ratios were derived from Cox proportional hazards models, adjusting for age, race, high school education, coverage by private medical insurance, pack-years of smoking, diabetes mellitus, and plasma interleukin-6 concentration. Comparisons were made with participants whose CMV IgG antibody concentration was ≤1.20 IU/mL.

known to be associated with high CMV antibody titer (8). However, the association between CMV and mortality remained significant after adjusting for cardiovascular disease. After we excluded women with cardiovascular disease from the study sample, there remained a suggestion, although not definitive evidence, of a higher mortality risk for women in the highest quartile of CMV antibody concentration. Thus, although residual confounding is still possible, the significantly higher mortality risk for women with a high CMV antibody concentration could not be attributed completely to cardiovascular disease. Indeed, if CMV infection were responsible for the onset of frailty or accelerated death, there must be certain intermediary pathophysiologic changes, whether measureable clinically or presently not definable or quantifiable, that eventually result in the manifestation of frailty (32) or accelerated death.

An alternative explanation can be offered for the association reported here of CMV IgG concentration with frailty and mortality. A higher frequency of CMV reactivation could be a bystander, rather than an initiator, of pathologic changes in human hosts. For example, production of tumor necrosis factor-α, as during the course of natural, non-CMV-related infections or inflammatory responses, could induce CMV reactivation (36). Therefore, the data presented in this paper are also consistent with the alternative hypothesis that CMV reactivation, reflected in the higher antibody concentrations, is a by-product of non-CMV-related pathophysiologic changes in frail older women or those experiencing accelerated death.

This study has several strengths. It utilized a prospective, well-characterized cohort of community-dwelling older women (19, 20). The definition of frailty utilized in this study is a well-validated measure, collected by using objective and standardized tests (14, 21). In addition, multiple statistical models utilized in the longitudinal analysis ensured robustness of our results. Rigorous adjustment for socioeconomic

factors minimized their potential confounding effects on the relation between CMV and the assessed outcomes. Finally, use of study-specific probability weights enables extrapolation of the results to a larger population (24).

There are potential limitations to this study. The exact duration of persistent CMV infection could not be determined by measuring CMV antibody concentration. Serologic diagnosis of primary infection was not possible because of limited serum availability. CMV reactivation, optimally measured in immunocompetent humans by detecting CMV DNA in urine (29), could not be determined because urine samples were not stored. Since a cellular response to CMV has been reported to be detected in seronegative individuals (37), underdiagnosis of CMV infection was possible but should not have affected the internal validity of these findings. It is unclear whether long-term cryopreservation might affect the stability of serum CMV IgG antibody, but no loss of titer was observed for serum antibodies cryopreserved for 5 years and measured by immunoassay (38). There might be differences between men and women in their immune response to CMV (39); the investigation should be repeated with men. Finally, the follow-up duration of 3 years and 5 years might not adequately capture the long-term effect of CMV infection on the development of incident frailty or mortality, respectively, in older women. Nevertheless, the results of the present study offer the first known generalizable longitudinal data on the effect of CMV infection on mortality and frailty development in immunocompetent older women.

In summary, we demonstrated an association between high CMV IgG antibody concentration and prevalent frailty among women with a high plasma IL-6 concentration, and between high CMV IgG antibody concentration and 5-year mortality risk, independent of potential confounders. The mechanisms underlying these associations remain to be elucidated. These findings provide a strong rationale for well-designed longitudinal population studies investigating the long-term clinical effects and pathogenic mechanisms of persistent CMV infection, the collective results of which can guide public health efforts on potential preventive and therapeutic strategies for this widely prevalent infection.

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